

Phase Shifting Interferometric Analysis of Protein Crystal Growth Boundaries and Convective Flows

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The objective of the proposed study is to obtain experimental evidence of several characteristics of a crystallizing protein solution and model their effects on the crystal growth process. The characteristics to be studied include: the presence of concentration gradients during the crystal growth, the extent of the boundary layer from the crystal face, and the effect of buoyancy driven convection on the growth. Whether such convection is able to produce flows capable of disturbing the attachment of macromolecules onto the crystal surface is still a matter of controversy among scientists in the field. Phase shifting interferometry (PSI) can provide significant insight to this issue. PSI will produce a visual confirmation of the concentration profile at the boundary layer and concentration values within the depletion region. PSI has the potential to offer a direct visualization of convective flows within proteinic crystallizing solutions. The importance of this matter is enhanced by the accepted fact within the field that the suppression of these convective effects is the only proven consequence of establishing a low gravity environment for protein crystals. On a recent Shuttle flight (USML-1), Dr. Larry de Lucas observed that the nucleation rates seemed lower and that the crystals were still in a growing regime at the end of the mission. The investigation of convective flows in relation to gravity as a parameter for protein crystallization is therefore of importance.

Protein crystals are grown to determine the three-dimensional structure of proteins. By

utilizing x-ray or neutron diffraction, the collected information allows the direct identification of active sites of the macromolecule, its conformation, and sequence of the amino acids. Sections of very large assemblies of proteins such as structural proteins or viruses can also be crystallized. Crystallization is therefore the starting point of any study aiming at the development of new drugs and the understanding of viral diseases. Crystallization techniques for proteins are now well known, but a biophysical understanding of the growth mechanisms is underdeveloped. This aspect of protein research needs to be expanded as the proteins being studied are more complex and their purification more costly.

Microgravity grown crystals of several proteins were found to be larger or diffracted with higher resolution than ones previously grown on the ground. These results tend to demonstrate that a reduction of gravity affects the interfacial growth mechanisms which are directly dependent upon the mass transport regime. Convection is known to play a significant role in the growth kinetics of inorganic crystals but its importance is still debated in relation to the crystallization of biological macromol-

ecules. There is little doubt about the existence of convective flows in proteinic solutions, but the flow rates they generate close to the crystal/solution interface and their effect on growth kinetics have not been quantified experimentally. The technique of PSI will allow us to determine these values and provide direct comparison between solutal flows in crystallizing solutions under various levels of gravity. An examination of the flows in the fluid and its correlation with crystal growth will strongly depict the role of microgravity in protein crystal growth.

Mach-Zehnder and Michelson-Morley interferometry will be used to examine growing protein crystals. Phase shifting will be accomplished using an electro-optic phase modulator or a piezo-electric mirror. PSI can provide a resolution of at least $1/100$ of a wavelength. Phase maps generated by the PSI will provide a correlation between crystal growth and convective flow in the growth fluid. Inorganic crystals and protein crystals have been grown. The optical system (fig. 137) is a phase shifting Michelson-Morley interferometer. The phase-shifting algorithm chosen to collect data uses five $\pi/2$ phase

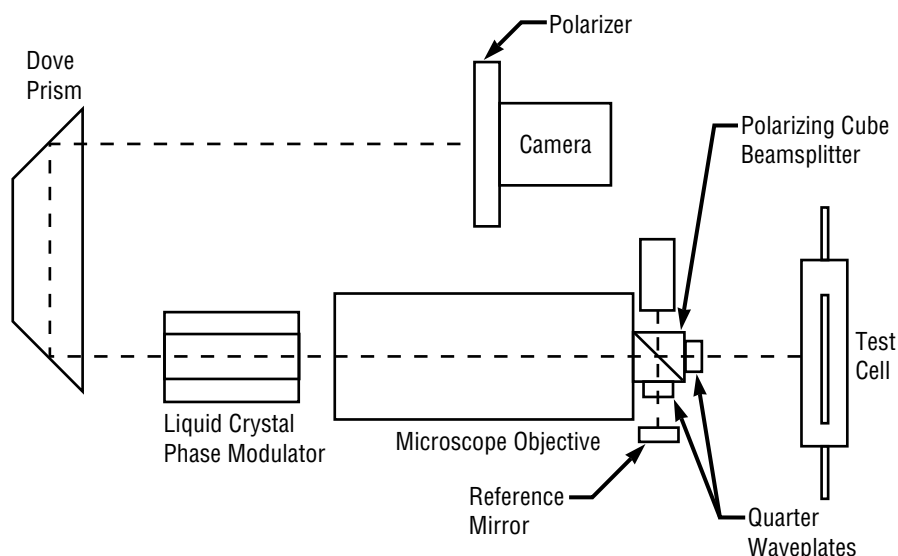


FIGURE 137.—Schematic of phase shifting interferometer.

shifts. By using $\pi/2$ phase shifts, the PSI algorithm becomes insensitive to phase shift errors. A computer program has been developed that controls the phase shifter, collects the interference data, and calculates the phase map. Initial experiments used sodium chlorate crystals to test the PSI. Strong concentration gradients are formed around sodium chlorate crystals when they grow. The next experiments will grow lysozyme protein crystals. The PSI system is also being developed into a flight system to be flown on the Russian Mir station next year. After the Mir experiments, ground-based protein crystal growth will be compared with the same type of crystals grown in micro-g. Variations of the PSI system and software developed at MSFC/NASA are being used by groups at the University of California at Riverside, CA, and at the De Montfort University in England.

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Biographical Sketch: William Witherow currently holds the position of AST, Basic Properties of Materials, at MSFC. He designs and fabricates optical data acquisition systems for various experiments. The areas of experiments include protein crystal growth, immiscible fluids studies, crystal growth, multicolor holography, phase-shifting interferometry, optical measurements of alloy solidification in a micro-g environment, and nonlinear optics measurements. His current work also includes image analysis, image digitization, and he also serves as the project scientist for the observable protein crystal growth facility. He holds a B.S. in engineering physics, 1977, from the University of Tennessee, Knoxville; and an M.S. in physics, 1981, from the University of Alabama in Huntsville. 